AMENDMENTS TO THE SPECIFICATION

IN THE SPECIFICATION:

Please replace paragraph [0026] on page 7, beginning on line 3 with "FIG. 2" and concluding with "...or the mitochondria." with the following amended paragraph:

[0026] FIG. 2 Shows schematic representations of example intracellular targeting sequences for use with single chain monoclonal antibody reagents. Targeting vectors direct expression of sFvs to either the cytoplasm, nucleus, endoplasmic reticulum, or the mitochondria. NLS, nuclear localization sequence, = DPKKKRKV (SEQ ID NO: 92). ER, endoplasmic reticulum localization sequence, = SEKDEL(SEQ ID NO: 93).

Please replace paragraph [0027] on page 7, beginning on line 7 with "FIG. 3" and concluding with "...or the mitochondria." with the following amended paragraph:

[0027] FIG. 3 Shows the pBTM116 yeast expression plasmid as an example vector used to construct antigen (X) "bait" strain fusions to screen the antibody fusion reagent library. The plasmid has a polylinker with the sequence GAATTCCCGGGGATCCGTCGACCTGCAG (SEQ ID NO:95).

Please replace the last paragraph on page 24, beginning on line 25 with "Preferred embodiments of the single chain..." and concluding with "... or V_H-$[(Gly)_4Ser]_3$-V_L." on line 4 of page 25, with the following amended paragraph:

Preferred embodiments of the single chain monoclonal antibody fusion reagents consist of an antibody light chain variable domain (V_L) and heavy chain variable domain (V_H) connected by a short flexible linker, preferably a peptide $[(Gly)_4Ser]_3$ (SEQ ID NO:96) which allows the molecule to assume a conformation that is capable of binding an antigen. Nicholls, PJ, Johnson, VG, Blanford, MD, Andrew, SM., J. Immunol. Methods, 165:81-91, 1993. Most preferably there is a short flexible linker between the two immunoglobulin variable domains, e.g., V_L-$[(Gly)_4Ser]_3$ (SEQ ID NO:96)-V_H; or V_H-$[(Gly)_4Ser]_3$ (SEQ ID NO:96)-V_L.

Please replace the paragraph that bridges pages 26 and 27, beginning "Another embodiment..." and concluding "... Embo J. 1:101-108, 1990." with the following amended paragraph:

Another embodiment of the present invention is nuclear expression for anti-transcription factor single chain monoclonal antibody fusion reagents. A nuclear-targeting version of an expression vector (FIG. 2) facilitates cloning of the immunoglobulin domain with 3 repeats of the nuclear localization signal (NLS) derived from SV40 T antigen (DPKKKRKV(SEQ ID NO: 92)) and a myc epitope tag at the C-terminus. Biocca, S, Nueberger, M S, Cattaneo, A. Embo J. 1:101-108, 1990.

Please replace the second paragraph on page 27 of the specification that begins on line 5 with "Targeting of single chain antibody . . ." and ends on line 14 with ". . . VEGF)." with the following paragraph:

Targeting of single chain antibody fusion reagents of the present invention to the endoplasmic reticulum is a contemplated embodiment to prevent secretion of specific proteins. A presently available endoplasmnic reticulum (ER) targeting vector allows for cloning of the antibody region in frame with a myc epitope tag followed by an ER retention signal (SEKDEL (SEQ ID NO: 93)). Munro, S, Pelham, RB. Cell 48:899-907, 1987. The utility of this embodiment is to prevent secretion of a protein that is normally secreted by sequestration/neutralization and/or retaining the target/fusion reagent complex in the endoplasmic reticulum. Anti-erbB2 and anti-VEGF are embodiment fusion reagents to block secretion of a transmembrane protein (epidermal growth factor (EGF) receptor with anti-erbB2) and a secreted protein (vascular endothelial growth factor (VEGF) with anti-VEGF).

Please replace the second paragraph on page 34 of the specification that begins on line 10 with "
The linkered . . ." and ends on line 13 with ". . .variable domains." with the following paragraph:

The linkered variable region PCR products are generated using the appropriate primers that have been fused to a sequence that when overlapped with the homologous sequences from the other chain variable region product will encode the $[(Gly)_4Ser]_3$ (SEQ ID NO:96) linker sequence between the two variable domains.

Please replace the paragraph on page 48 of the specification that begins with " The linkered . . ." and ends on line 13 with

". . . variable domains." with the following paragraph:

Generation of cDNA: PCR Amplification Total RNA is isolated for example from 3 human peripheral blood lymphocyte preparations (San Diego Blood Bank, Calif.) and portions of 4 human spleens using a SNAP™ Total RNA kit (Invitrogen, San Diego). The pooled total RNA is used in four separate first strand cDNA synthesis reactions and primed with one of the four constant region-specific primers (SEQ ID NO: 3-6). See Table I. These four oligonucleotides are specific to either the human heavy chain, IgM and IgG, or light chain, lambda and kappa, constant regions. The first strand reactions are performed using a cDNA Cycle Kit (Invitrogen) and subjected to two consecutive rounds of transcription. The heavy and light chain variable genes are PCR amplified from the cDNA using a mixture of family-specific human V-gene back primers and human germ-line J-segment forward primers (SEQ ID NO: 7-86). See Table I-III. The product from each PCR is run on a 1% agarose gel and purified using ~~Geneclean, Bio 101,~~ GENECLEAN® nucleic acid purification reagent (Bio 101), and then reamplified with similar primers containing restriction sites. See Tables I-III. These primer pairs add an ApaLI and NotI site to the light chain segments or an SfiL and SalI site to the heavy chain fragments.

Please replace the second paragraph on page 70 of the specification that begins on line 24 with "3. Prepare a petri dish . . ." and ends on page 71, line 2 with

The ". . .less than 30 min." with the following paragraph:

3. Prepare a petri dish for the reaction. In the lid place 1.5 ml Z buffer containing 15 ul of 50 mg/ml X-gal (Z buffer=60 mM Na_2HPO_4, 40 mM $Na_2H_2PO_4$, 10 mM KCl, 1 mM $MgSO_4$, pH 7.0). Lay 1 #1 ~~Whatman filter~~ WHATMAN® filter paper circle in the Z buffer, followed by the nitrocellulose filter with colonies facing up. Cover with the bottom of the dish and place at 30°C. If longer incubations are required for positive signals to be visualized, the petri dish should be placed in a humidified chamber. Strong interactions yield detectable color in less than 30 min.

Please replace the third paragraph on page 21 of the specification that begins on line 9 with "A preferred kit. . ." and ends on page 21, line 9 with ". . .See Tables I-III." with the following paragraph:

A preferred kit for screening a DNA construct library for a single chain monoclonal fusion reagent capable of binding a transcriptional associated biomolecule in vivo comprises pVP16Zeo (ATCC deposit # [[_____]] 98483) as DNA construct 3. Another contemplated embodiment of the kit provides a human sFv library integrated into DNA construct 3, preferrably pVP16Zeo. Another contemplated embodiment of the kit provides primers for human sFv library construction. In one embodiment primers may be selected from the group consisting essentially of SEQ ID NOs: 3-86 as described infra. See Tables I-III.

Please replace the second paragraph on page 31 of the specification that begins on line 8 with "Novel vectors that express . . ." and ends on page 31, line 17 with ". . . yeast and E.coli." with the following paragraph:

Novel vectors that express the single chain monoclonal antibody fusion reagents are also embodiments of the present invention. The vector pVP16Zeo, described infra, is a most preferred embodiment (FIG. 5) (ATCC access #[[_____]]98483). The pVP16Zeo library expression vector is most preferred for the construction and screening of single chain monoclonal antibody fusion reagent libraries, comprising zeocin selection to facilitate the isolation and production of single chain monoclonal antibody fusion reagents in yeast and E.coli. Generally, relatively small cloning vectors (under 5 kb) which have a convenient multiple cloning site as well as functional promoter (e.g. yeast ADH promoter) to drive expression of the heterologous sequence as well as efficient termination signals for 3' mRNA processing--are preferred for ease of manipulation in library construction. ~~Zeocin~~ Zeocin is preferred as a dual selectable marker in yeast and E.coli.

Please replace the third paragraph on page 46 of the specification that begins on line 16 with "Two peptide antigen. . ." and ends on page 46, line 21 with ". . . Cell, 74:205 (1993)." with the following paragraph:

Two peptide antigen strains, ATF-2FL and CREBIP-BOX were constructed (see Example I) as LexA DBD fusions in pBTM116 (ATCC access #[[_____]] 98483), to screen the antibody fusion reagent library. The peptide antigen (X) "bait" strains were constructed using the pBTM116 yeast expression plasmid for example (FIG. 3) which contains a Trp1 gene for selection in yeast and the DBD of Lex A with a downstream polylinker to allow generation of Lex A DBD/antigen X ("bait") fusion proteins. Vojtek, A. B., Hollenberg, S. M., Cooper, J. A., Cell, 74:205 (1993).

Please replace the fourth paragraph on page 46 of the specification that begins on line 25 with "The yeast expression library. . ." and ends on page 47, line 7 with ". . . See Example VIII." with the following paragraph:

The yeast expression library vector pVP16Zeo (ATCC access #[[_____]] 98483) is constructed from three parent constructs, pPICZB (Invitrogen, San Diego), pGBT9 (~~Clonetech~~ Clontech), and pVP16 (Vojtek, A. B., Hollenberg, S. M., Cooper, J. A., Cell, 74:205 (1993)), for the construction and screening of single chain monoclonal antibody fusion reagent libraries, comprising zeocin selection to facilitate the isolation and production of single chain monoclonal antibody fusion reagents. Selection in pVP16Zeo is based on a single selectable marker that confers resistance to the drug Zeocin in both Saccharomyces cerevisiae and E. coli. Collis,CM, Hall, RM. Plasmid 14:143-151, 1985; Wenzel, TJ, Migliazza, A, Ydesteensma, H, Vandenberg JA. Yeast 8:667-668, 1992. Zeocin selection is also compatible with either trp or leu selectable markers which may be used as "bait" plasmid markers. See Example VIII.

Please replace the fourth paragraph on page 76 of the specification that begins on line 17 with "The yeast expression library. . ." and ends on page 76, line 24 with ". . . plasmid markers." with the following paragraph:

The yeast expression library vector pVP16Zeo (ATCC access #[[_____]] 98483) was constructed from three parent constructs: pPICZB (Invitrogen, San Diego), pGBT9 (~~Clonetech~~ Clontech), and pVP16. Vojtek, A. B., Hollenberg, S. M., Cooper, J. A., Cell, 74:205 (1993). Selection in pVP16Zeo is based on a single selectable marker that confers resistance to the drug Zeocin in both ~~Saccharoniyces~~ Saccharomyces cerevisiae and E. coli. Collis,CM, Hall, RM. Plasmid 14:143-151, 1985; Wenzel, TJ, Migliazza, A, Ydesteensma, H, Vandenberg JA. Yeast 8:667-668, 1992. Zeocin selection is also compatible with either trp or leu selectable markers which may be used as "bait" plasmid markers.